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World J Gastroenterol 2003;9(6):1261-1264
World Journal of Gastroenterology
 ISSN 1007-9327

TT virus infection in patients with chronic hepatitis B and response of TTV to lamivudine

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Received: 2003-02-25 **Accepted:** 2003-03-16

Abstract

AIM: To investigate the responses of TT virus (TTV) and hepatitis B virus (HBV) to a long-term lamivudine therapy.

METHODS: Sixteen patients infected with both TTV and HBV were treated with lamivudine 100 mg daily for 30 months. Blood samples were drawn at the beginning of the therapy and subsequently at month 3, 6, 9, 12 and 30. Serum TTV was quantified by real time PCR and serum HBV was detected by hybridization assay and nested polymerase chain reaction.

RESULTS: TTV infection was detected in 100 % of HBV-infected patients. Loss of serum TTV DNA after one year of treatment occurred in 1/16 (6 %) patients. At the end of therapy, TTV DNA was positive in 94 % of them. The decline of HBV viremia was evident at 3 months after therapy and the response rate was 31 %, 44 %, 63 %, 50 % and 50 % at month 3, 6, 9, 12 and 30, respectively.

CONCLUSION: TTV replication is not sensitive to lamivudine and is highly prevalent in HBV-infected patients.

Garcia JM, Marugan RB, Garcia GM, Lindeman MLM, Abete JF, Terron SC. TT virus infection in patients with chronic hepatitis B and response of TTV to lamivudine. *World J Gastroenterol* 2003; 9(6): 1261-1264
http://www.wjgnet.com/1007-9327/9/1261.asp

INTRODUCTION

TT virus (TTV) was recently discovered in a patient with post-transfusional hepatitis of unknown etiology[1]. Its genome is a circular, single-stranded DNA of negative polarity, which shares similarities with the members of the Circoviridae family[2]. In contrast to DNA viruses, TTV isolates exhibit a high level of genetic heterogeneity[3]. TTV presents an extreme diffusion of active infection throughout the world[4]. Its presence in blood products could indicate that TTV can be transmitted through transfusion[5]. Although it has been proposed that TT virus might be responsible for the small proportion of acute and chronic forms of hepatitis that still remain unsolved, no other illness has yet been attributed to the virus.

Hepatitis B virus (HBV) causes transient and chronic infections of the liver, which may progress to cirrhosis and eventually to hepatocellular carcinoma (HCC)[6]. Interferon (IFN)-alpha is the only approved drug for the treatment of chronic HBV infection, but the recent registration of lamivudine, a dideoxycytidine analogue that inhibits both the HIV and HBV reverse transcriptases, has provided new perspectives for the treatment of chronic HBV infection[7, 8]. Coinfection of TTV and HBV is commonly seen because both viruses share the same transmission routes such as blood transfusion[9]. In previous studies, IFN therapy has been reported effective against TTV[10], but the possible susceptibility of the virus to lamivudine treatment has not yet been investigated. Thus, the aims of this study were: (1) to know the impact of TTV in patients with chronic hepatitis B; (2) to investigate the response of TTV to lamivudine therapy; and (3) to evaluate whether the outcome of long term lamivudine therapy in chronic hepatitis B is influenced by a TTV coinfection.

MATERIALS AND METHODS

Patients

The study group consisted of 16 patients (12 males and 4 females; mean age: 52.62 years; range: 33 and 66 years) with chronic dual HBV and TTV infection. The diagnosis of chronic hepatitis was made on the basis of clinical and histological results. Patients included 15 cases of liver cirrhosis and 1 hepatocarcinoma. In 9 patients the transmission route was unknown, 2 had a history of blood transfusion, 2 developed HBV infection after liver transplantation, 1 had primary biliary cirrhosis and 2 were addicts to alcohol. At the beginning of the therapy, all patients were positive to HBV DNA by solution hybridization assay and had elevated alanine aminotransferase (ALT) levels that were 3 times of the upper normal limit (normal range: 5-45 IU/L). Nine patients were hepatitis B e antigen (HBeAg) positive, 5 had antibodies against HBeAg (anti-HBe) and 2 patients were negative to both HBeAg and anti-HBe.

Ten patients underwent liver transplantation; 8 of them for HBV-related liver cirrhosis. The remaining 2 patients without evidence of HBV infection before allograft developed a *de novo* post-transplant HBV infection.

Lamivudine (Glaxo Wellcome, UK) was given orally to all patients at a dose of 100 mg daily for 30 months. Blood samples were taken at the baseline time and subsequently were obtained at month 3, 6, 9, 12 and 30. To evaluate the effects of lamivudine, levels of ALT, TTV DNA and HBV DNA and viral serological markers were evaluated at each time. HBV complete responders to lamivudine therapy were defined as patients showing clearance of serum HBV DNA by nested polymerase chain reaction (PCR) and normal ALT levels at 30 months of treatment. All patients gave written informed consent before the enrollment in the study, which was approved by the ethics committees of the hospital.

Detection and quantification of TTV DNA

TTV DNA quantification was carried out with a real time PCR by the SYBR Green approach using primers targeting the untranslated region (UTR) of the viral genome: forward primer T801: 5' -GCTACGTCACTAACCACG-3' , position 6 to 25; reverse primer T935: 5' -CTGCGGTGTGTAAACTCACC-3' , position 185 to 204[11]. Total DNA was purified from 200 μl of serum using the High Pure Viral Nucleic Acid Kit (Roche Diagnostic, Mannheim, Germany) and eluted in a final volume of 50 μl. Real time PCR was done using 2 μl of the eluted DNA with 0.2 μM of each primer in 23 μl 2×SYBR Green PCR mix (Qiagen). The cycling conditions were: 95 ℃ for 10 min to activate the DNA polymerase followed by 45 cycles of amplification: 95 ℃ for 15 sec, 62 ℃ for 30 sec and 72 ℃ for 30 sec.

HBV markers

Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and antibodies to HBsAg, HBeAg and hepatitis B c antigen (HBcAg) were determined by immunoassay (EIA; Abbott Laboratories, N Chicago, IL). Serum HBV DNA levels at the beginning of lamivudine therapy were quantified using Abbott hybridization assay. Viral DNA during the following time points was detected by nested PCR[12].

Statistical analysis

Statistical analysis was performed using Student' s *t* test. Data were analyzed with the computer program SPSS (SPSS Inc., Chicago, IL, USA). A probability (*P*) value of less than 0.05 was considered statistically significant.

RESULTS

Detection and response of TTV to lamivudine therapy

Sixteen patients doubly infected with TTV and HBV were monitored for levels of both viruses in serum, at selected time points, during the lamivudine treatment by real time and nested PCR methodology, respectively. Of the 16 patients, serum TTV DNA could be detected in all of them by real time PCR at the beginning of lamivudine therapy, with TTV values that ranged between 8.7×10^3 to 1.9×10^8 genomes per ml of serum (mean: 5.7×10^8 genomes/ml). After 30 months of lamivudine treatment, 15/16 patients (94 %) still had TTV DNA in serum and TTV values ranged between 3×10^4 to 4.2×10^8 genomes per ml of serum (mean: 4.3×10^7 genomes/ml). The patient who became negative to TTV DNA lost this marker after 12 months of treatment and he still remained TTV DNA negative until the end of therapy. The TTV DNA value of this patient at baseline time point was 8×10^4 viral genomes per ml of serum. However, this patient did not respond to the lamivudine therapy and he was serum HBV DNA positive at each time point. With respect to the 15 positive TTV DNA patients, at the end of the treatment and relative to baseline levels, 1 patient (7 %) showed unchanged serum TTV DNA levels, 6 patients (40 %) had 6 times of reduction of serum TTV load and 8 cases (53 %) presented 8 times of increase of the levels of TTV DNA in serum.

Changes in serum TTV DNA concentration with respect to HBV non-responsive and responsive patients to lamivudine treatment were also analyzed. It was found that there was no statistically significant difference when basal and final serum samples were compared between both groups (Table 1) or when basal and final serum samples were compared in the same group (Table 2).

Finally, serum TTV DNA values in patients with or without liver transplants were evaluated as well. Once again, there were not significant differences when baseline and final serum samples were compared between non-transplanted and transplanted patients (Table 3) or when baseline and final serum samples were compared between non-transplanted or transplanted patients (Table 4).

Table 1 Comparison of serum TTV DNA concentration changes before treatment and at the end of treatment

	Non-responders (*n*=7)	Responders (*n*=8)	*P*[a]
Before treatment	4.6×10^7	8.0×10^7	0.66
End of treatment	1.1×10^8	3.4×10^7	0.29

TTV DNA concentration was expressed as viral genomes / ml of serum. [a]Student's *t*-test, HBV non-responsive patients *vs.* responsive patients to lamivudine therapy.

Table 2 Changes of TTV DNA concentration in HBV non-responsive and responsive patients to lamivudine therapy

	Before treatment	End of treatment	*P*[a]
Non-responders (*n*=7)	4.6×10^7	1.1×10^8	0.60
Responders (*n*=8)	8.0×10^7	3.4×10^7	0.55

TTV DNA concentration was expressed as viral genomes / ml of serum. [a]Student's *t*-test, before *vs* at the end of treatment.

Table 3 Comparison of serum TTV DNA concentration changes before treatment and at the end of treatment

	Non-transplanted (*n*=5)	Transplanted (*n*=10)	*P*[a]
Before treatment	1.2×10^7	8.9×10^7	0.22
End of treatment	8.8×10^6	1.0×10^8	0.07

TTV DNA concentration was expressed as viral genomes / ml of serum. [a]Student´s *t*-test, non-transplanted *vs.* transplanted patients.

Table 4 Changes in TTV DNA concentration in non-transplanted and transplanted patients

	Before treatment	End of treatment	*P*[a]
Non-transplanted (*n*=5)	1.2×10^7	8.8×10^6	0.59
Transplanted (*n*=10)	8.9×10^7	1.0×10^8	0.87

TTV DNA concentration was expressed as viral genomes / ml of serum. [a]Student´s *t*-test, before *vs* at the end of treatment.

HBV response to lamivudine treatment

At the baseline time point, HBV DNA values ranged between 4.2×10^9 and 8.4×10^{11} DNA genomes per ml of serum (mean: 1.2×10^{11} genomes/ml). The decline of HBV viremia was clearly evident at month 3 after therapy and the response rate was 31 %, 44 %, 63 %, 50 % and 50 % at month 3, 6, 9, 12 and 30, respectively. When compared between responsive and non-responsive patients, the vesponders had almost 4 times the value of the non-responder ones but the difference was not significant (2.6×10^{11} *vs* 7×10^{10} genomes per ml of serum, respectively). Also, there was no significant difference in baseline of ALT levels between responsive and non-responsive patients (117 IU/L *vs.* 123 IU/L, respectively). However, differences were statistically significant in post-treatment ALT levels between responsive and non-responsive patients (37 IU/L *vs* 128 IU/L, respectively; *P*=0.03). Of the 5 HBeAg positive patients, 2 seroconverted to anti-HBe by month 12. The other 3 patients still remained HBeAg positive after 30 months of lamivudine therapy. In the 2 HBeAg and anti-HBe negative patients, they

developed anti-HBe by month 3.

With respect to the 10 liver transplant recipients, 4 patients (40 %) started to receive lamivudine treatment after recurrent allograft re-infection. In these cases, serum HBV DNA was still detectable after 30 months of therapy. However, in those 6 patients who started lamivudine administration before liver transplantation as a prophylaxis regimen, 5 (50 %) lost viral DNA at month 3 of treatment, and then they underwent liver allograft and remained HBV DNA negative throughout the therapy. By contrast, the remaining patient developed a recurrent HBV infection at month 6 of post-transplant.

DISCUSSION

Since TTV was discovered a few years ago, many studies have been done trying to assess whether it causes liver disease; however, there is still a poor understanding of its molecular properties and pathogenic potential.

This study shows, in agreement with other groups, that TTV infection is chronic and characterized by the continued presence of high virus loads in serum with wide variations ranging between 10^3 and 10^8 genomes per ml[13, 14]. Over a period of 30 months, 96 % of the patients presented high TTV DNA levels; however, in some individuals, viremia levels fluctuated extensively while they remained essentially constant in others.

Different epidemiological studies have clearly indicated that TTV can behave as a transmissible blood-borne virus sharing common transmission routes with the hepatitis viruses. Then, coinfection of TTV is frequently observed in patients with chronic hepatitis B[9]. One important finding in our study is that 100 % of patients infected with HBV were TTV DNA-positive by real-time PCR. These results suggest that the prevalence of TTV infection is very high in the HBV-infected population. However, our results are in disagreement with other studies that reported TTV DNA rates between 15 % and 36 %[15-17]. One explanation for this result might be due to the different primers used for the detection of TTV DNA; we used a set of primers that already showed 92 % positivity to TTV in healthy adults in Japan[11]. Another possible explanation why these patients were TTV positive is their histological status: 94 % of them presented cirrhosis. A TTV well-recognized feature is that TTV infection is chronic and tends to last many years, so it could be that TTV infection is more prevalent in patients with advanced HBV-associated liver disease than in those with stable disease.

However, the most novel and interesting information that emerged from this study is that lamivudine treatment in a regimen of 100 mg daily and for a period of 30 months did not inhibit TTV replication in patients coinfected with HBV and TTV. Only one individual (6 %) of the 16 patients enrolled in the study became serum TTV DNA negative after 12 months of therapy. This observation could suggest that this patient lost TTV in a spontaneous way and not by effect of lamivudine on TTV replication.

With respect to the effect of lamivudine treatment of chronic hepatitis B, this study confirms earlier reports where HBV infection responded positively to lamivudine treatment[18-20]. The response of HBV to lamivudine treatment was not affected with the concurrent TTV infection. This observation can be explained by two reasons. Firstly, the changes observed in ALT values during the lamivudine treatment period were correlated with the change of HBV DNA in chronic hepatitis B; it seemed that ALT dynamic was unrelated to TTV viremia. Secondly, TTV viremia was not related to different HBV DNA levels, so TTV did not seem to interfere with HBV replication. Thus, in agreement with many other published studies, TTV may lack clinical association with liver disease in these patients[21, 22].

Finally, it has been suggested a relationship between an increased TTV viral load and host immunological disorders[23]. In immunosuppressed patients, such as recipients of liver transplants, TTV viremia could be higher than that in individuals without liver allografts. However, we did not find any significant difference in TTV viral load when both groups were compared, even if transplanted patients had always higher TTV titers. Based on our results it seems that immune system is not involved in elevated TTV viremia in HBV patients, although more extensive studies need to be performed to prove this hypothesis.

In conclusion, during the lamivudine therapy for chronic hepatitis B, disappearance of TTV does not occur in the majority of the HBV-infected patients which supports the interpretation that lamivudine does not inhibit TTV replication. Moreover, this study shows the highly prevalence of TTV infection in patients with chronic hepatitis B, but without any effect on the course of HBV infection.

REFERENCES

1 **Nishizawa T**, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* 1997; **241**: 92-97

2 **Miyata H**, Tsunoda H, Kazi A, Yamada A, Khan MA, Murakami J, Kamahora T, Shiraki K, Hino S. Identification of a novel GC-rich 113-nucleotide region to complete the circular, single-stranded DNA genome of TT virus, the first human circovirus. *J Virol* 1999; **73**: 3582-3586

3 **Hijikata M**, Takahashi K, Mishiro S. Complete circular DNA genome of a TT virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. *Virology* 1999; **260**: 17-22

4 **Abe K**, Inami T, Asano K, Miyoshi C, Masaki N, Hayashi S, Ishikawa K, Takebe Y, Win KM, El Zayadi AR, Han KH, Zhang DY. TT virus infection is widespread in the general populations from different geographic regions. *J Clin Microbiol* 1999; **37**: 2703-2705

5 **Matsumoto A**, Yeo AE, Shih JW, Tanaka E, Kiyosawa K, Alter HJ. Transfusion-associated TT virus infection and its relationship to liver disease. *Hepatology* 1999; **30**: 283-288

6 **Lee WM**. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745

7 **Eron JJ**, Benoit SL, Jemsek J, MacArthur RD, Santana J, Quinn JB, Kuritzkes DR, Fallon MA, Rubin M. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. *N Engl J Med* 1995; **333**: 1662-1669

8 **Dienstag JL**, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 1995; **333**: 1657-1661

9 **Kao JH**, Chen W, Chen PJ, Lai MY, Chen DS. TT virus infection in patients with chronic hepatitis B or C: influence on clinical, histological and virological features. *J Med Virol* 2000; **60**: 387-392

10 **Nishizawa Y**, Tanaka E, Orii K, Rokuhara A, Ichijo T, Yoshizawa K, Kiyosawa K. Clinical impact of genotype 1 TT virus infection in patients with chronic hepatitis C and response of TT virus to alpha-interferon. *J Gastroenterol Hepatol* 2000; **15**: 1292-1297

11 **Takahashi K**, Hoshino H, Ohta Y, Yoshida N, Mishiro S. Very high prevalence of TT virus (TTV) infection in general population of Japan revealed by a new set of PCR primers. *Hepatol Res* 1998; **12**: 233-239

12 **Lindh M**, Gonzalez JE, Norkrans G, Horal P. Genotyping of hepatitis B virus by restriction pattern analysis of a pre-S amplicon. *J Virol Methods* 1998; **72**: 163-174

13 **Lefrere JJ**, Roudot-Thoraval F, Lefrere F, Kanfer A, Mariotti M, Lerable J, Thauvin M, Lefevre G, Rouger P, Girot R. Natural history of the TT virus infection through follow-up of TTV DNA-positive multiple-transfused patients. *Blood* 2000; **95**: 347-351

14 **Oguchi T**, Tanaka E, Orii K, Kobayashi M, Hora K, Kiyosawa K. Transmission of and liver injury by TT virus in patients on maintenance hemodialysis. *J Gastroenterol* 1999; **34**: 234-240

15 **Lai YC**, Hu RT, Yang SS, Wu CH. Coinfection of TT virus and response to interferon therapy in patients with chronic hepatitis B or C. *World J Gastroenterol* 2002; **8**: 567-570
16 **Naoumov NV**, Petrova EP, Thomas MG, Williams R. Presence of a newly described human DNA virus (TTV) in patients with liver disease. *Lancet* 1998; **352**: 195-197
17 **Tanaka H**, Okamoto H, Luengrojanakul P, Chainuvati T, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. Infection with an unenveloped DNA virus (TTV) associated with posttransfusion non-A to G hepatitis in hepatitis patients and healthy blood donors in Thailand. *J Med Virol* 1998; **56**: 234-238
18 **Lai CL**, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61-68
19 **Leung NW**, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, Lim SG, Wu PC, Dent JC, Edmundson S, Condreay LD, Chien RN. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001; **33**: 1527-1532
20 **Leung N**. Liver disease-significant improvement with lamivudine. *J Med Virol* 2000; **61**: 380-385
21 **Gimenez-Barcons M**, Forns X, Ampurdanes S, Guilera M, Soler M, Soguero C, Sanchez-Fueyo A, Mas A, Bruix J, Sanchez-Tapias JM, Rodes J, Saiz JC. Infection with a novel human DNA virus (TTV) has no pathogenic significance in patients with liver diseases. *J Hepatol* 1999; **30**: 1028-1034
22 **Forns X**, Hegerich P, Darnell A, Emerson SU, Purcell RH, Bukh J. High prevalence of TT virus (TTV) infection in patients on maintenance hemodialysis: frequent mixed infections with different genotypes and lack of evidence of associated liver disease. *J Med Virol* 1999; **59**: 313-317
23 **Touinssi M**, Gallian P, Biagini P, Attoui H, Vialettes B, Berland Y, Tamalet C, Dhiver C, Ravaux I, De Micco P, De Lamballerie X. TT virus infection: prevalence of elevated viremia and arguments for the immune control of viral load. *J Clin Virol* 2002; **21**: 135-141

Edited by Xu XQ and Zhu LH